

## **29<sup>th</sup> Symposium**

### **Croatian Society of Medical Biochemistry and Laboratory Medicine**

#### **Laboratory analysis of extravascular specimens and body fluids**

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## Laboratory analysis of extravascular specimens and body fluids

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While whole blood, serum and plasma are extensively studied sample types which are commonly used in laboratories worldwide, some other matrices like various body fluids, are not so well known and are only recently increasingly gaining importance. The reason behind this are international quality standards and accrediting bodies which now clearly require that some specific quality characteristics are tested and known for each unique sample type: precision, accuracy, linearity, measurement range, sample collection, sample stability, interferences, reporting, clinical utility and some other. These requirements and quality specifications have raised many issues and challenges for laboratories. Therefore, the aim of this symposium is to provide an overview of the current knowledge and understanding of the sample characteristics, analytical specificities and clinical utility of samples such as pleural, peritoneal and synovial fluid as well as urine. Some new emerging protein biomarkers and analytical techniques such as microchip technology, proteomic and lipidomic analysis, gas chromatography and mass spectrometry and molecular techniques will also be discussed.

We hope that this symposium will address some frequently raised questions and provide useful guidance for professionals in laboratory medicine as well as other professions in the healthcare.

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## Paracentesis - aspiration of ascitic fluid from peritoneal cavity (when, why and how?)

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Ascites is abnormal accumulation of fluid in the free abdominal (peritoneal) cavity. There are many causes of ascites, including cirrhosis of the liver, abdominal cancer, congestive heart failure, constrictive pericarditis, liver veno-occlusive disease, inflammatory causes, peritoneal dialysis, pancreatitis, abdominal trauma, and *etc.* The majority of patients presenting with a clinical manifestation of ascites have liver cirrhosis. Clinical manifestations of the ascites are distended (swelling) abdomen, abdominal discomfort and pain, dyspnoea, fever, shortness of breath, and *etc.* The gold standard for ascites diagnosis is ultrasound of the abdomen. If causes of ascites may not be determined based on the history, examination, and review of laboratory data and imaging studies it is necessary to do the paracentesis. It involves sterilizing of the area on the abdomen and, with the guidance of ultrasound, inserting a needle into the abdominal cavity and withdrawing fluid for further analysis. Except for diagnostic purposes (to determine the cause of ascites), paracentesis is also performed in cases where a large amount of abdominal fluid significantly compromise breathing and in situations where the therapeutic response to diuretics is poor. Macroscopic appearance of ascites may indicate the disease, *e.g.* "blurred" ascites indicates the development of secondary bacterial peritonitis, "milky like" appearance is characteristic for chylous ascites (triglycerides > 200 mg/dL), pink (haemorrhagic) appearance suggest a higher amounts of erythrocyte (> 10 000/mm<sup>3</sup>). Ascitic fluid analysis include: cell counts and differential for leukocytes (inflammatory sign); ascitic fluid total protein and albumin (to distinguish exudate

from transudates which is it clinically important because it is indicative of the underlying pathological process), glucose, lactate dehydrogenase (LD), amylase, triglycerides, bilirubin, tumor markers (e.g. CEA, CA19-9, CA125) and others. The cytological examination of ascitic fluid is helpful in identifying and distinguishing the subgroups of malignancy-related ascites. Microbiological analysis determines whether microorganisms are present in fluids.

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## Synovial fluid - when, why and how?

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Joint aspiration and synovial fluid (SF) analysis is one the most commonly performed diagnostic procedures in modern clinical orthopaedics. Although knee is the most common joint requiring aspiration, all non-axial joints are accessible for obtaining SF. The technique requires good knowledge of anatomical landmarks and the use of ultrasound can be helpful in those smaller or/and less accessible joints. If performed under aseptic conditions and with sterile equipment the procedure carries only minimal risk for the patient. Analysis of aspirated SF is very helpful in differential diagnosis of arthritis and provides definitive diagnosis in septic arthritis, crystalopathies and periprosthetic joint infections (PJI). Macroscopic appearance of aspirate is very useful since it provides initial information and directs differential diagnosis. In general, the more inflammation is the present in the joint, the higher is volume of the aspirate, the higher is cell count and the higher is turbidity (muddiness of the liquid). Percentage of polymorphonuclear (PMN) leukocytes correlates well with degree of inflammation, and predominance of eosinophils may be seen in parasitic infestations and other hypereosinophilic conditions. Number of leukocytes in aspirate is current golden standard

in diagnosis of PJI, and the cut-of value is set at >2000 in mm<sup>3</sup> for definitive diagnosis of PJI. Appropriate stains and cultures, along with antibiogram should be performed in all SF samples. Detection of monosodium-urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals is considered crucial in diagnosis of gout and pseudogout (calcium pyrophosphate dihydrate deposition disease), respectively. In conclusion, it is obvious that SF aspiration and analysis can be of major help in differential diagnosis of many joint pathologies. However, more research is warranted to further improve our understanding of the true value of SF inspection, cell counts, specificity and sensitivity of microbiological assays, crystal detection, biochemical markers and cytological features.

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## Verification of automated haematology analyser for body fluid analysis (guidelines, recommendations)

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The diagnostic value of the cellular components in body fluids has been evaluated in many inflammatory diseases. Despite its many limitations, manual microscopy is still considered the gold standard for detecting and differentiating cells in body fluids. However, development of the automated haematology analysers has led to major improvements in body fluid analysis for cellular composition. These analysers offer improved accuracy, precision and efficiency compared with manual microscopy methods. Considering the importance of cell enumeration in some clinical conditions (e.g. bacterial meningitis and cellular enumeration of cerebrospinal fluid), it is essential that the laboratory provide precise, accurate and reproducible results, thus imposing on laboratory professionals

some new challenges - how to properly and efficiently perform the verification of automated cell counter for body fluids. The literature about this relevant topic is fairly scarce. The 2006 Clinical and Laboratory Standards Institute (CLSI) document H56-A Body Fluid Analysis for Cellular Composition; Approved Guideline provides recommendations for standardizing the collection and transport of body fluids, numeration and identification of cellular components, and guidance for qualitative and quantitative assessment of body fluid.

Although the recommendations are clear and comprehensive, they lack some guidance for conducting a proper verification of body fluid analysis on routine haematology analyser. In 2014 the International Council for Standardization in Hematology (ICSH) published the "Guidelines for the verification and performance of automated cell counters for body fluids" to assist laboratories to plan and execute the verification of their automated cell counters for automated body fluid counts. These guidelines represent valuable tool for every laboratory professional who has determined to cope with this demanding, yet challenging area of laboratory medicine. What is perhaps lacking are uniform, consistent and clearly defined quality requirements. There is definitely room for a literature, as well as for a knowledge upgrade.

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## **Pre-analytical phase in analysis of pleural, peritoneal and synovial fluids**

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In aetiology of effusion, every formation of extravascular fluid is a pathological process and samples are taken by punctation of body cavities (punctates). Extravascular body fluid sample is generally considered irreplaceable because sampling is often very invasive. Punctates are highly instable and therefore represent very challenging sample for laboratory analysis. Because of high

content of fibrinogen, there is a quick formation of coagulum. Additionally, each temperature change is affecting the total count of blood cells and metabolites (especially glucose). The most frequent laboratory analysis of body fluid samples include: haematological analysis (total white and red blood cells count), concentration of glucose, total proteins and albumins, as well as determination of enzyme activity such as lactate dehydrogenase and amylase. The recommended tube for haematology analysis is the EDTA anticoagulant test tube, while for biochemical analysis the recommended tube is heparin tube and heparin syringe for blood gas analysis. Common demand for each of these tests is the time of delivery no longer than 15 minutes after sampling in room temperature. According to our experience, the most common mistake in pre-analytic phase of body fluid testing is inadequate test tube with inadequate anticoagulant. In literature sample transport in inadequate temperature is mentioned as the most common cause of mistake in pre-analytic phase of punctate analysis. To ensure that body fluid sample is suitable for intended purpose there is a need for active dialog with people responsible for collecting samples and following the laboratory instructions for body fluid collection and transportation.

The aim of this lecture is to give a review of the most common pre-analytical issues in analysis of pleural, peritoneal and synovial fluids, and possibly provide some specific solutions.

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## **Urinary tract infections in the emergency department - diagnostic guidelines**

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Urinary tract infection (UTI) is a common clinical condition in the emergency department (ED)

which clinical presentations vary from the simple cystitis to urosepsis. Management of UTIs in the ED is very challenging due to high patient turnover, limited history and lack of culture and susceptibility test results. Because a urine culture is usually not available for 24–48 hours after an ED visit, diagnosis and treatment decisions are based on symptoms, physical findings, and some laboratory results. Although, diagnosis of UTI by clinical criteria has very high diagnostic error rates of 30–50%, the use of diagnostic algorithms does not completely eliminate uncertainty.

Urinalysis is valuable and the most frequently used diagnostic tool for the evaluation of potential urinary tract infection, and accurate interpretation of urinalysis results is a key in diagnosis and proper antibiotics treatment. However, because urinalysis is so commonly ordered in the emergency department, there is serious concern that misinterpretation may lead to overtreatment with antibiotics. Therefore, it is very important to understand both the strengths and the limitations of the diagnostic test for UTIs. Blood tests and further test beyond urinalysis and urine cultures are needed only to diagnose complicated UTI as well as for inpatients that develop UTIs.

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## Standardization of qualitative urinalysis in Croatia

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Qualitative urinalysis is one of the most commonly used routine laboratory tests and is often a first step in the diagnostic pathway of urinary tract infections, proteinuria, haematuria and some other conditions. Unfortunately, wide heterogeneity among dipstick manufacturers results in various dipsticks sensitivity, specificity and measurement range expressed as categories.

In Croatia, 24 dipstick brands are available on the market and used interchangeably in 195 clinical laboratories. Comprehensive analytical verification showed that 12 most commonly used dipsticks are not sufficiently comparable. Besides low level of agreement, there is substantial variability in analytical performance. Consistent with previous studies, we verified that dipsticks do not accurately detect glucose and proteins regarding to quantitative measurements. Another analytical limitation is unsatisfying precision for few parameters on the dipsticks, especially pH.

The diversity of manufacturers is also manifested in the number of measurement fields for each parameter. Urine dipsticks have more strip pads than the number of categories recommended by the Croatian Chamber of Medical Biochemists (CCMB) for reporting results of dipsticks urinalysis. Therefore, it is up to each laboratory to define concentration ranges for all reporting categories. The results of questionnaire sent to 195 Croatian laboratories (response ratio was 71%) showed large inconsistency in categories assigned to concentrations. The fact that 30% false negative results were observed for leukocytes, and 21% for glucose, represents possible risk of unrecognized urinary tract infections and early diabetes.

Considering worldwide utilization of urine dipsticks, such heterogeneity and lack of standardization may lead to diagnostic errors, patient misclassification, and consequently jeopardize the patient safety.

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## New biomarkers - an insight into the future

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One of the proposed definitions of the term "biomarker" describes it as "biological property whose in vitro measurement is useful for the prevention,



diagnosis, prognosis, treatment, and follow-up of diseases". The Test Evaluation Working Group (TEWG) of the EFLM prepared set of interactive tools that enable evidence-based evaluation of novel biomarkers.

Peritoneal fluid represents valuable matrix for determination of biomarkers like adenosine deaminase (ADA) in differentiation between tuberculosis peritonitis and peritoneal carcinomatosis. Ascites is sort of "ecosystem" that modifies together with course of malignant disease, changing its cell content and "molecular signature", thus giving valuable information to the clinician. Use of microfluidic chip technology in ascites analysis facilitates monitoring of ovarian cancer treatment.

Lipidomic analysis of synovial fluid (SF) from osteoarthritis and rheumatoid arthritis patients showed significant alteration in composition when compared to normal controls. Osteoarthritis severity could be estimated by detection of proportion of mesenchymal stem cells in synovial fluid. Detection techniques like GC/TOF MS enable comparison of metabolic profiling of SF in population of patients with rheumatoid arthritis and other in-

flammatory diseases. Recent studies on SF showed that S100A8/A9 and IL-35 could be used as diagnostic and prognostic biomarkers in RA.

Pleural fluid (PF) concentrations of IL-6, IL-8 and VEGF are increased in acute graft rejection of lung transplant. C-reactive protein, soluble triggering receptor expressed on myeloid cells (sTREM) and lipopolysaccharide-binding protein (LBP) in PF have discriminatory power in assessment of infectious effusions. Beyond routinely used parameters in analysis of PF, there are publications pointing to biomarkers like ADA, cholesterol/triglyceride, interferon-gamma, NT-proBNP, tumor markers, amylase, creatinine, complement, antinuclear antibodies and rheumatoid factor in detection of aetiology of disorder.

Novel promising biomarkers in analysis of urine are osteopontin, KIM-1, IL-18 and NGAL.

Implementation of novel biomarkers into guidelines requires well-designed studies which follow defined checklists for evaluation of biomarkers.

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